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The Art and Science of Diagnosing *Mycoplasma pneumoniae* Infection

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Mycoplasma pneumoniae causes a significant burden of disease in children as both upper and lower respiratory tract infections (URTIs and LRTIs). A positive pharyngeal polymerase chain reaction (PCR) or serology for *M. pneumoniae* can be found in 4–39% of children hospitalized with community-acquired pneumonia (CAP).[1] Since the introduction of the pneumococcal conjugate vaccine, *M. pneumoniae* has been reported to be the most common bacterial cause of CAP among hospitalized U.S. children.[2]

M. pneumoniae is transmitted by respiratory droplets through close contact. The incubation period can be as long as 1–3 weeks. *M. pneumoniae* infection is generally mild and self-limiting. However, patients of every age can develop severe CAP or extrapulmonary manifestations. The lack of a cell wall makes *M. pneumoniae* resistant to cell wall synthesis inhibitors such as β-lactam antibiotics. Antibiotics effective against *M. pneumoniae* include macrolides, tetracyclines and fluoroquinolones.[3] However, a Cochrane review[4] concluded that there is insufficient evidence to draw any definitive conclusions about the efficacy of antibiotics for *M. pneumoniae* LRTI in children. Macrolides are extensively used worldwide, and this has led to alarming resistance rates among *Streptococcus pneumoniae* and *M. pneumoniae*.[5] Reported macrolide-resistant *M. pneumoniae* (MRMP) prevalence is particularly high in Asia with over 90% in some regions, resulting in therapy refractory *M. pneumoniae* CAP.[5] Efficacy data and tailored prescription of antibiotic treatment are needed to minimize further selection of MRMP. Unfortunately, currently, there is no single diagnostic method that confirms active *M. pneumoniae* infection in CAP.

This review focuses on the diagnosis of *M. pneumoniae* infections in children and discusses clinical and microbiologic features that may help identifying *M. pneumoniae* as the cause of CAP.

CLINICAL ASSESSMENT

Clinical assessment – the art in diagnosing *M. pneumoniae* infection

The term "walking pneumonia" had been introduced to denote the mild form of CAP in most patients with *M. pneumoniae* infection. These patients can generally be managed in primary care. Physicians often rely solely on clinical suspicion in such cases.

EPIDEMIOLOGY

M. pneumoniae occurs endemically worldwide. Infections can be observed throughout the year, but tend to be more common in summer and early fall. Epidemic peaks can be observed every 3–7 years, whereas climate and geography may not be relevant.[5,6] Outbreaks of *M. pneumoniae* infections have been reported within families, schools, institutions and military bases. Clinicians should be particularly aware of *M. pneumoniae* as potential cause of CAP during *M. pneumoniae* epidemics. *M. pneumoniae* infections can occur in all ages. However, *M. pneumoniae* CAP is reported to be most frequent among school-age children 5–15 years of age.[1,2,5]

SIGNS AND SYMPTOMS

In addition to the presentation at school-age, children with CAP due to *M. pneumoniae* have been found to present with a significantly longer duration of fever

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compared with other CAP patients.[7] A fast-and-frugal clinical decision tree provided a rapid probability estimate of the cause of CAP in children: determining the preceding duration of fever combined with the age of the child allowed identification of patients at high risk for *M. pneumoniae* CAP, that is, children with CAP who have had fever > 2 days and who were > 3 years of age.[7] The decision tree placed 72% of all patients with *M. pneumoniae* infection into the high-risk group.

Apart from fever, clinical signs and symptoms of *M. pneumoniae* infection vary widely. The commonest symptoms are a sore throat and a (typically nonproductive) cough. Other symptoms may be the absence of wheeze and the presence of chest pain.[8] Symptoms typically develop over several days and an intractable cough often persists for weeks to months. However, another Cochrane review[8] concluded that *M. pneumoniae* infection cannot be reliably diagnosed based on clinical symptoms alone. Nevertheless, a combination of age and clinical features rather than specific findings may aid clinicians in identifying patients at high risk for *M. pneumoniae* CAP.

EXTRAPULMONARY MANIFESTATIONS

Additional clinical features of *M. pneumoniae* infection include extrapulmonary manifestations, which can affect almost every organ, including the skin and the nervous, hematologic, cardiovascular and musculoskeletal system.[9] These manifestations are caused either by direct local effects of *M. pneumoniae* after dissemination or indirect immune-mediated effects.

Skin manifestations occur in up to 25% of all *M. pneumoniae* infections, including mainly nonspecific exanthems, urticaria, and (less commonly) erythema nodosum. There are also rare but distinct pediatric *M. pneumoniae*-associated skin disorders such as erythema multiforme, Stevens-Johnson syndrome, and *M. pneumoniae*-associated mucositis.[9]

Encephalitis and Guillain-Barré syndrome constitute the most severe neurologic manifestations, where *M. pneumoniae* infection is thought to be causative in up to 10% and 21% of patients, respectively.[10,11] We recently demonstrated that *M. pneumoniae* triggers antibodies against the major myelin antigen galactocerebroside (GalC), and showed that anti-GalC IgG is critical for the development of Guillain-Barré syndrome following *M. pneumoniae* infection.[11] Because the detection rate of *M. pneumoniae* by PCR in cerebrospinal fluid (CSF) of *M. pneumoniae* encephalitis patients is low (0–14%), a significant proportion of the cases may be immune-mediated as well.[10] In fact, we also demonstrated anti-GalC IgG antibodies in serum and CSF of patients with encephalitis[12] and severe Guillain-Barré syndrome with additional CNS symptoms,[13] which suggests that these antibodies are also involved in the development of *M. pneumoniae*-associated CNS disease.

The presence of extrapulmonary manifestations in children with CAP significantly increases the probability of *M. pneumoniae* infection.

LABORATORY PARAMETERS

CAP patients with uncomplicated *M. pneumoniae* infection often have normal or only slightly raised absolute leukocyte and neutrophil counts, as well as lower C-reactive protein levels than children with CAP caused by other bacterial organisms.[5,7]

CHEST RADIOGRAPH

The radiographic presentation of "atypical" pneumonia due to *M. pneumoniae* is extremely variable. Bilateral, diffuse interstitial infiltrates are common, pleural effusions can occur, but none of the radiographic findings associated with *M. pneumoniae* CAP are specific.[1]

NONRESPONSE TO EMPIRICAL β-LACTAM ANTIBIOTICS

The British Thoracic Society guidelines[1] recommend amoxicillin as first choice for oral antibiotic therapy in children with suspected bacterial CAP. They also advise that macrolide antibiotics may be added at any age in case of very severe disease or if there is no response to first-line empirical treatment. In children with CAP who do not recover within a few days as would be expected in viral infection, and who do not respond to β-lactam antibiotics, clinicians should consider *M. pneumoniae* CAP.

DIAGNOSTIC TESTS

Diagnostic tests – the science in diagnosing *M. pneumoniae* infection

Children with moderate-to-severe CAP and/or presence of risk factors (underlying disease or immunodeficiency) should be referred to secondary care for further assessment.[1] Microbiologic diagnosis should be attempted in those children. Current guidelines[1,3] recommend PCR and serologic tests to diagnose *M. pneumoniae* infections. An overview of diagnostic tests is shown in Table 1.

PCR

PCR is considered as the new "gold standard" with a superior sensitivity and shorter turnaround time than culture. Nucleic acid amplification techniques for the detection of *M. pneumoniae* DNA or RNA differ in the choice of target genes used (e.g., P1 gene, 16S rDNA, 16S rRNA etc.), (PCR vs. isothermal amplification techniques), and detection formats (conventional vs. real-time, monoplex vs. multiplex). In the recent past, research focused on the evaluation of commercially available tests,[5,14] multiplex assays,[14] and strain typing methods.[5]

Importantly, like many other respiratory pathogens, *M. pneumoniae* can be carried in the upper respiratory tract of asymptomatic children. Detection rates in children without symptoms of a respiratory tract infection vary from 3% or less to 56%.[2,5,15] It appears that the mere presence of *M. pneumoniae* in the upper respiratory tract may not necessarily indicate respiratory disease.[15]

CULTURE

Culture is not used for routine diagnosis because it is labor-intensive, needs special enriched broth or agar media, and the incubation period can take up to 3 weeks.

RAPID ANTIGEN TEST

Rapid antigen tests have a limited sensitivity because of a detection limit of approximately 1×10^3 colony-forming units (CFU)/ml.[5,16] Although they have a lower sensitivity than PCR, the detection time is faster and only less-trained staff is required compared with culture.

SEROLOGY

The sensitivity of specific serologic tests depends on the time point of the first serum sample and on the availability of paired sera collected ≥ 2 weeks apart to evaluate seroconversion and/or ≥ 4-fold antibody titer increase ("gold standard"). Specific serum immunoglobulin (Ig) M can be detected within 1 week after initial infection and about 1–2 weeks before IgG.[10] Reinfection in adults can lead directly to an IgG response and may lack production of IgM. Specific serum IgA rises, peaks and decreases earlier than IgM, but is less frequently detected.[15]

The previously used serologic tests are complement fixation tests, particle agglutination assays, and immunofluorescent assays, which were based on crude *M. pneumoniae* antigen extracts. Since *M. pneumoniae* contains large amounts of glycolipids that elicit cross-reactive antibody responses (manuscript submitted), enrichment for adhesion protein P1 or protein extracts without glycolipid antigens has been used to improve the test performance of enzyme immunoassays (EIAs).

Intriguingly, one study reported that IgM as well as IgG and IgA could be detected by EIA in single serum samples of asymptomatic *M. pneumoniae* PCR-positive children.[15] The antibody response in these children

TABLE 1. Overview of Diagnostic Tests for *M. pneumoniae*

Method	Test	Target/Antigen	Antibodies/Cells	Specimen(s)	Performance	Diagnostic Value
Direct detection of *M. pneumoniae*	PCR	Different target genes (e.g., P1 gene, 16S rDNA, 16S rRNA)	—	Respiratory specimen, other bodily fluids or tissues	High sensitivity, high specificity	Routine
	Rapid antigen test	Different antigens (e.g., adhesion protein P1[16])	—	Respiratory specimen	Moderate-high sensitivity, moderate-high specificity	(Routine)*
	Culture	—	—	Respiratory specimen	Low sensitivity, high specificity	Advanced
Nonspecific serologic tests for *M. pneumoniae*	Cold agglutinin test ("bedside test")	Erythrocytes (I antigen)	Cold agglutinins (IgM)	Serum	Low sensitivity, low specificity	(Routine)†
Specific serologic tests for *M. pneumoniae*	CFT	Antigen extracts with glycolipids and/or proteins	Igs (no discrimination between isotypes)	Serum	Less sensitive and less specific than EIA	(Routine)‡
	PA		IgM and/or IgG	Serum	Sensitivity and specificity comparable with EIA	(Routine)‡
	IFA				Less sensitive and less specific than EIA (subjective interpretation)	(Routine)‡
	EIA	Proteins (e.g., adhesion protein P1) and/or glycolipids	IgM, IgG, IgA	Serum	Moderate-high sensitivity, Moderate-high specificity	Routine
	Immunoblotting				High sensitivity, high specificity (confirmatory assay)§	Advanced
Specific ASC response for *M. pneumoniae*	ELISpot	Proteins (e.g., adhesion protein P1) and/or glycolipids	IgM, IgG, IgA ASCs	Blood (PBMCs)	High sensitivity, high specificity (unpublished data)	Advanced

*Not available worldwide.

†Historical test: cold agglutinins are IgM antibodies that target the I antigen of human erythrocytes during *M. pneumoniae* infection and precipitate when a blood sample is placed in an anticoagulated tube on ice for around 30 seconds; replaced by specific serologic tests.

‡Largely replaced by EIA.

§Dumke *et al*. Diagn Microbiol Infect Dis 2012;73:200–203.

CFT, complement fixation test; ELISpot, enzyme-linked immunospot assay: IFA, immunofluorescent assay; Ig, immunoglobulin; PA, particle agglutination; PBMC, peripheral blood mononuclear cell.

Table adapted from Meyer Sauteur *et al*.[10]

may simply reflect a previous encounter with *M. pneumoniae* and is not necessarily related to the concurrent presence of *M. pneumoniae* in the upper respiratory tract.

Overall, no single diagnostic test or combination of tests is capable of differentiating carriage from infection in a clinically relevant time frame.

ANTIBODY-SECRETING CELL RESPONSE

The humoral immune response is highly specific for the infecting pathogen. However, the use of convalescent sera is not helpful in clinical settings, because of the time delay that is inevitable when waiting for a titer increase. The specific B cell response is more rapid and short-lived, and thus an optimal target for determining infectious etiology in CAP patients.[17] It can be detected by measuring antibody-screening cells (ASCs) with an enzyme-linked immunospot assay. A recent study found that *M. pneumoniae*-specific ASCs indeed circulate in peripheral blood only during CAP, while serum antibodies remain at high levels over months (unpublished data). The detection of ASCs could therefore potentially serve as a future diagnostic tool discriminating *M. pneumoniae* infection from carriage.

In conclusion, clinicians need to be aware of the implications and clinical significance of a positive PCR and/or serology test result for *M. pneumoniae*. Rather than relying on diagnostic test results alone, clinicians need to interpret these results in combination with clinical features and a lack of response to β-lactam antibiotics.

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